



# Genome Sequences of Soft Rot-Causing *Pectobacterium* Isolates from Different Vegetables

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**ABSTRACT** Eleven *Pectobacterium* strains were isolated from soft rot-diseased vegetables. Here, we report their genome sequences and characteristics. Five isolates were found to be *Pectobacterium versatile*, while the other six were determined to be *Pectobacterium brasiliense*.

Soft rot disease of plants is a deterioration of plant tissues, resulting in smelly, mushy, and inedible fruits and vegetables. The bacteria that cause soft rot are among the most important plant pathogens (1), with *Pectobacterium* and *Dickeya* species representing the leading disease-causing agents in the field and postharvest (2). They employ plant cell wall-degrading enzymes, including: pectin lyases, proteases, and cellulases, which macerate plant cells and tissues (3). Once disease symptoms occur, treatment is impossible, so prevention of this disease is key. We have isolated 11 soft rot-causing bacteria from various rotting vegetables, all of which were *Pectobacterium* strains; here, we provide their draft ( $n = 8$ ) and complete ( $n = 3$ ) genome sequences.

The soft rot phenotype was confirmed by swabbing rotted material from vegetables onto sterilized carrot slices (4) and, after incubation at 30°C in a moist chamber for 48 h, isolating the bacterial communities from the diseased carrots on Luria-Bertani (LB) agar. Pure cultures from the LB plates were then individually swabbed onto sterilized carrot slices and incubated as before to confirm the soft rot phenotype. Examples of soft rot caused by these isolates are shown in Fig. 1. Sanger sequencing of 16S rRNA gene PCR products using common 16S rRNA primers, 27F (5'-AGAGTTTGATCMTG GCTCAG-3') and 1492R (5'-GTTACCTTGTTACGACTT-3'), for amplification and an NCBI BLASTn search against the NCBI nonredundant (nr) database identified the isolates as *Pectobacterium* species (5). For genome sequencing, DNA extraction was performed using the Nanobind CBB (cells, bacteria, blood) big DNA kit (Circulomics, Baltimore, MD). Sequencing was conducted using Illumina MiSeq 250-bp read length paired-end sequencing at the Iowa State University DNA Facility, generating between 0.67 million and 1.7 million reads per sample and 3.03 Gbp of total sequence data (Table 1). Library preparation was performed using the NEBNext Ultra II FS kit with standard parameters. FastQC v0.11.9 was used to assess the quality of the reads (note: default parameters were used for all software unless specified otherwise) (6). Bases with a quality score of below 20 were trimmed and adapter sequences were removed using BBDuk v37.36 with the following options: "ref=adapters.fasta ktrim=r ordered k=23 hdist=1 mink=11 tpe tbo qtrim=w trimq=20 minlen=75" (7). Only reads greater than 75 bp after trimming were used to generate initial genome assemblies, using SPAdes v3.14.1 with the "--careful" option (8). Based on average nucleotide identities (ANI) between the isolates, calculated using JSpeciesWS (9), three strains were chosen for additional sequencing with Oxford Nanopore GridION technology to obtain closed genomes, with the same DNA samples used in the Illumina run. Library preparation was performed using the SQK-LSK109 kit

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**FIG 1** Examples of soft rot caused by the sequenced *Pectobacterium* isolates on various vegetables. Potatoes (A), bell peppers (B), celery (C), radishes (D), and carrots (E) were inoculated with 10  $\mu$ L of an overnight culture of each isolate and incubated at 30°C in a moist chamber. Pictures were taken at 24 h (celery), 48 h (radish and carrot), and 72 h (potato and bell pepper) after inoculation. Dark, wet, and mushy spots are symptoms of soft rot. The scale bars in each image equal 1 cm.

with barcoding kit EXP-NBD104 with standard parameters. Approximately 400,000 Nanopore reads were generated, covering 8.01 Gbp total (Table 1). The Illumina MiSeq and Nanopore reads were used to generate hybrid genome assemblies using either SPAdes with the “--careful” option (SR1 and SR10) or Unicycler v0.4.8 (SR12) (10). Annotation of the assembled genomes was performed through the PATRIC database and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (11, 12).

The genome sizes ranged from 4.85 to 5.08 Mbp, the number of contigs ranged from 1 to 71, and the GC content was approximately 52% (Table 1). The ANI results grouped the isolates into two clusters: isolates SR1 to SR4 (within-group ANI, >99.9%) and isolates SR5 to SR8, SR10, and SR11 (within-group ANI, >99.9%). Further ANI grouping found isolates SR1 to SR4 and SR12 to be most similar to *Pectobacterium versatile* CFBP6051 (GenBank accession number [GCA\\_004296685.1](#); ANI, >97.3%) and isolates SR5 to SR8, SR10, and SR11 to be most similar to *Pectobacterium brasiliense* PcbHPI01 ([GCA\\_001429565.2](#); ANI, >98.1%). No plasmids were identified in the isolates; all have type III secretion systems, multiple pectin lyases, cellulases, proteases, iron uptake systems, and flagellar genes. This work provides the basis for further experiments on these agriculturally and economically important plant pathogens.

**Data availability.** The *Pectobacterium* sequences have been deposited in GenBank under the BioProject accession number [PRJNA767613](#). The GenBank and SRA accession numbers for each isolate are listed in Table 1.

**TABLE 1** *Pectobacterium* isolates and genome characteristics

Isolate	Taxonomy	Genome size (Mbp)	No. of contigs	GC content (%)	Source	GenBank accession no.	Raw reads (SRA accession no.)	No. of raw Illumina reads	Illumina sequence data (Mbp)	No. of Nanopore reads	Nanopore sequence data (Gbp)	Nanopore $N_{50}$ (kbp)
SR1	<i>Pectobacterium versatile</i>	5.08	1	51.9	Carrot	<a href="#">CP084656</a>	<a href="#">SRR16683343</a> , <a href="#">SRR16683344</a>	1,211,266	302.8	106,461	2.178	40.73
SR2	<i>Pectobacterium versatile</i>	5.02	66	52	Carrot	<a href="#">JAIXMD000000000</a>	<a href="#">SRR16683352</a>	705,578	176.4			
SR3	<i>Pectobacterium versatile</i>	5.02	71	52	Carrot	<a href="#">JAIXMC000000000</a>	<a href="#">SRR16683351</a>	1,114,156	278.5			
SR4	<i>Pectobacterium versatile</i>	5.02	68	52	Potato	<a href="#">JAIXMB000000000</a>	<a href="#">SRR16683350</a>	1,117,632	279.4			
SR5	<i>Pectobacterium brasiliense</i>	4.85	31	52	Pepper	<a href="#">JAIXMA000000000</a>	<a href="#">SRR16683349</a>	961,960	240.5			
SR6	<i>Pectobacterium brasiliense</i>	4.85	35	52	Squash	<a href="#">JAIXLZ000000000</a>	<a href="#">SRR16683348</a>	1,185,922	296.5			
SR7	<i>Pectobacterium brasiliense</i>	4.85	49	52	Pumpkin	<a href="#">JAIXLY000000000</a>	<a href="#">SRR16683347</a>	1,331,128	332.8			
SR8	<i>Pectobacterium brasiliense</i>	4.86	53	52	Squash	<a href="#">JAIXLX000000000</a>	<a href="#">SRR16683346</a>	1,679,466	419.9			
SR10	<i>Pectobacterium brasiliense</i>	4.89	1	52	Squash	<a href="#">CP084655</a>	<a href="#">SRR16683342</a> , <a href="#">SRR16683345</a>	673,258	168.3	173,470	3.169	36.04
SR11	<i>Pectobacterium brasiliense</i>	4.85	33	52	Pumpkin	<a href="#">JAIXLW000000000</a>	<a href="#">SRR16683341</a>	917,650	229.4			
SR12	<i>Pectobacterium versatile</i>	5.08	1	52	Coleslaw	<a href="#">CP084654</a>	<a href="#">SRR16683353</a> , <a href="#">SRR16683354</a>	1,220,496	305.1	120,189	2.666	42.35

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